REMARKS

Applicants request respectfully that the allowability of the claims be reconsidered in view of the above amendments and the following remarks.

Status of the Claims

The Examiner's Action addressed Claims 1, 7, 11, 14, 15, and 18 to 32. Claims 1, 7, 11, 14, 15, 20 to 29 and 31 have been amended. No claims have been added or cancelled. Accordingly, the claims pending presently for examination are Claims 1, 7, 11, 14, 15, and 18 to 32.

Discussion of the Amendments

Claim 1 has been amended to emphasize that applicants' claimed process starts with a DNA complex consisting essentially of DNA complexed with cationic lipds/polymers and then reacts the cationic lipids/polymers *in this complex* with the reagents citraconic anhydride or N-hydroxysuccinimide acetate to convert the cationic surface potential of the DNA complex to a neutral or net anionic surface potential. Claim 1 was also amended to correct the spelling of N-hydroxysuccinimide acetate.

Dependent Claims 7, 11, 14, 15, 20 to 29 and 31 have been amended to ensure proper antecedent basis.

No new matter has been added.

Discussion of the Invention

Applicants' invention is directed to a process for making a stable colloid that includes a complex of DNA and lipids or polymers. In the art, it is known that DNA can form complexes with lipids in which the lipids surround the DNA and sequester it. Similarly, it is known that DNA can form complexes with polymers. Because DNA is anionic, the most efficient way of forming such a complex is to use cationic lipids or cationic polymers. The cationic lipids or cationic polymers electrostatically interact with the DNA to form a complex. An undesired feature of such a complex, however, is that the complex has a cationic surface potential. In *in vivo* use, such a cationic surface potential causes anionic proteins to be attracted to the complex and cause it to be rapidly opsonized within the cell.

Given the above, it is known to modify the complex such that the cationic surface potential is reduced, removed or reversed such that the complex has a neutral or anionic surface potential to decrease opsonization of the complexes. Various methods are known in the art for accomplishing this. Such methods are described in Monahan et al. and in Trubetskoy et al. (both cited by the Examiner). In Monahan et al., N-hydroxysuccinimide ester or citraconic anhydride is used in the creation of anionic polymers which are then used to form an anionic envelope around the initial cationic envelope (the anionic polymers interact electrostatically with the cationic polymers of the initial envelope). Thus a two envelope complex is formed. Trubetskoy et al. also describes generally the formation of a second anionic envelope around the initial cationic envelope. Another method is described in Semple et al. In this method, the cationic lipid envelope is made using lipids which become anionic or neutral depending upon the pH of the surrounding environment. If the pH of the surrounding environment is high, hydrogen atoms on the lipids on the exterior surface of the envelope are released, thus converting the lipids on the exterior of the envelope

into anionic or neutral lipids. The lipids on the interior are not exposed to the surrounding environment and thus remain cationic and thus can continue to associate with the anionic DNA sequestered in the complex.

Applicants' development is novel and non-obvious in that, unlike Monahan et al. and Trubetskoy et al., it does not require the use of separate anionic polymers to form a second complex or envelope around the cationic complex of DNA and cationic lipids/polymers. Rather, like Semple et al., applicants modify the exterior surface lipids/polymers of the cationic lipid/polymer-DNA complex so that the surface lipids/polymers become netural or anionic. Unlike Semple et al., however, applicants' method does not require a change in the pH of the surrounding environment. Rather, N-hydroxysuccinimide acetate (NHS acetate) or citraconic anhydride (CCA) is used to react with the cationic lipids/polymers on the surface of the complex, thus converting the exterior lipids/polymers into anionic or neutral form. As the above reactions result in the addition of a chemical moiety to the lipids/polymers and not the release of a hydrogen atom (as in Semple et al.) and as a further anionic envelope is not added (as in Monahan et al. and Trubetskoy et al.), not only is applicants' method novel and non-obvious over the prior art, but applicants' colloid is structurally different from the colloids of the prior art and novel and non-obvious thereover as well.

Discussion of the Examiner's
Section 102(e) Rejection of Claims 18 to 23 and 30 Based on Monahan et al.

The Examiner has rejected Claims 18 to 23 and 30 as being anticipated under Section 102(e) by Monahan et al. (U.S. Patent No. 6,379,966). The Examiner asserts that absent evidence to the contrary, since claim 18 is drawn to a stable colloid regardless of its method of preparation, if the prior art compound reads on a process which involves the production of a stable colloid, the claim is unpatentable even though the prior art product was made by a different process.

Although the Examiner is correct in stating that the patentability of product-by-process claims is based on the product, "the structure implied by the process steps should be considered when assessing the patentability of product-by-process claims over the prior art, especially . . . where the manufacturing process steps would be expected to impart distinctive structural characteristics to the final product." M.P.E.P. § 2113. That is exactly the case here. While Monahan et al. and applicants' claimed product are both stable colloids, applicants' claimed product has distinctive, novel, and nonobvious structural characteristics imparted by the process as defined by Claim 1. The Examiner must consider the structural characteristics of the DNA complex imparted by applicants' novel and nonobvious process.

In the present case, because the process of amended Claim 1 first starts with a DNA complex of DNA complexed with cationic lipds/polymers and then reacts the cationic lipids/polymers *in this complex* with the reagents citraconic anhydride or N-hydroxysuccinimide acetate, the resulting colloid contains DNA complexes in which the lipids/polymers *complexed with the DNA* contain side groups derived from the reaction with citraconic anhydride or N-hydroxysuccinimide acetate as shown, for example, in Figures 4 and 7. Claim 1 has also been amended to recite that the colloid comprises "a DNA complex consisting essentially of DNA complexed with cationic lipids or cationic polymers." Accordingly, applicants' claimed DNA complexes include DNA complexed with lipids/polymers, but exclude other materials that would materially affect the basic and novel characteristics of the complex. See M.P.E.P. § 2111.03.

The colloid of Monahan et al. does not contain DNA complexes in which the polymers *complexed with the DNA* contain side groups derived from the reaction with citraconic anhydride or N-hydroxysuccinimide acetate. Moreover, Monahan et al.

discloses a colloid with DNA "particles" in which the cationic polymers complexed with the DNA are enveloped by an outer layer of anionic polymers. (See Monahan et al. at column 23, lines 56 to 59). As described by Monahan et al., this outer layer of anionic polymers materially affects the DNA "particles" by making the particle "negatively charged" and thus increasing "the stability of [the] DNA particles in serum." *Id.*

Accordingly, the colloid formed by the process of amended Claim 1 is significantly structurally different from that of Monahan et al. in that the colloid of Monahan et al. does not contain DNA complexes in which the polymers complexed with the DNA contain side groups derived from the reaction with citraconic anhydride or N-hydroxysuccinimide acetate. For this reason alone, Claim 18 is novel and nonobvious over Monahan et al. Moreover, as claim 1 has been amended to recite that the DNA complex of the colloid consists essentially of DNA complexed with lipids/polymers which have been reacted with citraconic anhydride or N-hydroxysuccinimide acetate, the colloid of Claim 18 formed by the process of Claim 1 would exclude any colloids that include DNA complexes having an outer envelope of anionic polymers as disclosed in Monahan et al.

In the present Action, the Examiner ignores the differences between the DNA complexes of the claimed invention and Monahan et al. Instead, the Examiner asserts that the colloid of the present invention, being a "stable colloid", is the same as that of Monahan et al. Just as all prior art hammers would not anticipate a claim drawn to a hammer made by a novel process which imparts novel structural characteristics to the hammer, all "stable colloids" do not anticipate a novel colloid that is prepared by a novel process. In particular, the process of Claim 1 provides a DNA complex consisting essentially of DNA complexed with lipids/polymers. Thus, the product-by-process of Claim 18 would include such DNA complexes. Similarly, the stable colloid

of Monahan et al. comprise DNA complexes. Accordingly, inasmuch as applicants' DNA complex differs from that of Monahan et al. as discussed above, the colloid which comprises it must necessarily be different from the corresponding colloid of Monahan et al. as well.

In view of the above, applicants request respectfully that the Examiner withdraw the anticipatory rejection of Claims 18 to 23 and 30.

Discussion of the Examiner's Section 103(a) Rejection
Based on Semple et al., Monahan et al., and Trubetskoy et al.

The Examiner has rejected Claims 1, 7, 11, 14, 15, 18 to 23, and 28 to 32 as being rendered obvious under Section 103(a) by Semple et al. (U.S. Patent No. 6,287,591) taken with Trubetskoy et al. (US 2003/0026841) and Monahan et al. (U.S. Patent No. 6,379,966).

Semple et al. is directed to a composition comprising lipid-therapeutic agent particles comprising a lipid portion and a therapeutic agent. The lipids of the particles can be present in both a charged and an uncharged form depending on the pH of the environment. For example, Semple et al. discloses that the lipid-therapeutic agent particles can be formed at a lower pH with cationic amino lipids in the presence of nucleic acids. The surface charge of the newly formed particles can then be neutralized by increasing the pH of the medium to a level above the pKa of the amino lipids present, i.e., to physiological pH or higher. (See Semple et al. at column 9, lines 15 to 27).

Accordingly, applicants' claimed process distinguishes over Semple et al. in that Claim 1 recites that the cationic surface potential of the DNA complex is converted to a neutral or net anionic surface potential by reacting the cationic lipids or

the cationic polymers in the DNA complex with a reagent selected from the group consisting of citraconic anhydride and N-hydroxysuccinimide acetate. Semple et al. does not disclose reacting citraconic anhydride or N-hydroxysuccinimide acetate with the lipid in the particle or complex to convert the surface potential to neutral or net anionic. As discussed below, neither Trubetskoy et al. nor Monahan et al. remedy this deficiency in Semple et al.

Trubetskoy et al. is directed to a process for delivery of a polyion to a cell. Trubetskoy et al. discloses adding polyanions to *preformed* DNA/polycation complexes (i.e., recharging). (See Trubetskoy et al. at paragraph 52). As such the polyanions of Trubetskoy et al. form an anionic envelope around the DNA/polycation complexes. Trubestskoy et al. does not disclose reacting citraconic anhydride or N-hydroxysuccinimide acetate with the polycation in the complexes to convert the surface potential of the complex from cationic to neutral or net anionic.

Monahan et al. is directed to a process for delivering a polynucleotide complex into an extavascular parenchymal cell of a mammal. Monahan et al. discloses the use of citraconic anhydride to react with a poly-L-lysine to render it anionic and the use of the resulting anionic polymer in the formation of an anionic envelope around a DNA-cationic polymer complex. (See Monhan et al. at column 23, lines 57 to 59 and column 25, lines lines 38 to 65). Monahan et al. also discloses the use of NHS esters as a crosslinker to link multi-valent oligopeptides of glutamic or aspartic acid to form cleavable anionic polymers. These resulting anionic polymers are also used to form an envelope around the DNA-cationic polymer complex. (See Monahan et al. at column 15, lines 27 to 38). Monahan et al. does not disclose reacting citraconic anhydride or N-hydroxysuccinimide acetate with the polycation in the complexes to convert the surface potential of the complex from cationic to neutral or net anionic.

For this rejection, the Examiner relies on the Action dated March 9, 2005. In that Action, the Examiner argues that one skilled in the art would have been motivated to modify the surface of a cationic lipid/DNA complex such as those employed by Semple et al. to ensure that the surface of these charged lipid/DNA particles have an overall neutral or negative charge to improve the *in vivo* circulation of the complexes and to improve gene transfer efficiency as evidenced by the teaching of Trubetskoy et al. and Monahan et al. The Examiner further asserts that one skilled in the art would have been motivated by Trubetskoy et al. to modify the DNA-cationic lipid complexes of Semple et al. by adding anionic compounds and been further motivated by Monahan et al. to create such anionic compounds by reacting cationic polymers with citraconic anhydride or NHS ester.

"To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure." M.P.E.P. § 2143.

Here, the prior art references cited by the Examiner do not teach or suggest all the claim limitations and there is no suggestion or motivation to combine the references as asserted by the Examiner.

The references do not teach or suggest all the claim limitations

As amended, Claim 1 clarifies that the applicants' process involves first providing a DNA complex consisting essentially of DNA complexed with cationic lipids or cationic polymers in which the DNA complex has a cationic surface potential, and converting the cationic surface potential of the DNA complex to a neutral or net anionic surface potential by reacting the cationic lipids or the cationic polymers *in the DNA complex* with a reagent selected from the group consisting of citraconic anhydride and N-hydroxysuccinimide acetate. None of the references discloses reacting any reagent with the lipids or polymers already complexed with DNA to reduce, remove or reverse the cationic surface potential of the DNA complex.

As discussed above, Semple et al. simply teaches changing the pH of the medium to neutralize the positive charges on the lipids in the DNA complex. Trubetskoy et al. and Monahan et al. teach a "recharging" step wherein anionic polymers are used to form an envelope around a DNA/cationic polymer complex. While Monahan teaches that citraconic anhydride can be used to neutralize polycationic polymers, none of the references teaches or suggests reacting a reagent, much less citraconic anhydride and N-hydroxysuccinimide acetate, with the cationic lipid or cationic polymer already complexed with the DNA.

There is no suggestion or motivation to combine the references

"Obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so." M.P.E.P. § 2143.01. Here, there is no such motivation.

Each of the references cited by the Examiner discloses developments by which the inventors had already addressed the problem of cationic DNA complexes. In Semple et al., the inventors use lipids that are cationic at a relatively low pH and are neutralized at physiological pH. In Monahan et al. and Trubetskoy et al., the inventors use anionic polymers to form an envelope around cationic DNA complexes to "recharge" such complexes. Accordingly, there would be no reason for one of skill in the art to look to modify the developments of these references, that is, there is no reason to combine their disclosures, to come up with the process recited in amended Claim 1.

For example, Semple et al. discloses a relatively simple way to package DNA in a lipid-complex that is initially cationic to facilitate DNA uptake and to neutralize the surface charges of the lipid by changing the pH of the medium. Thus, one of skill in the art would not be motivated to react a reagent with the lipids of the DNA complex of Semple et al. to neutralize the positive charges if the positive charges will be neutralized when the complexes are exposed to a physiological pH.

"The mere fact that references <u>can</u> be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination." M.P.E.P. § 2143.01. Here, there is no such desirability as the prior art discloses other developments to address the problem addressed by applicants' claimed process. It is only by using knowledge gleaned from applicants' disclosure that one of skill in the art would be motivated to combine these references. This, however, cannot be the basis for a *prima facie* case of obviousness. M.P.E.P. § 2145.

Moreover, using a reagent to neutralize the positive charge of a lipid-DNA complex would change the principle of operation of Semple et al. "If the proposed modification or combination of the prior art would change the principle of operation of

the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious." M.P.E.P. § 2143.01. Here, Semple et al. operates by changing the pH of the medium in which the complexes are present, e.g., the amino groups of the lipids are deprotonated as the pH is raised to a physiological pH. By reacting a reagent, such as citraconic anhydride or N-hydroxysuccinimide acetate, with these amino groups, the amino groups are neutralized and thus cannot be deprotonated when exposed to a higher pH. Monahan et al. and Trubetskoy et al. operate by forming an anionic layer around the cationic DNA-lipid/polymer complex. By reacting a reagent, such as citraconic anhydride or N-hydroxysuccinimide acetate, directly with the lipid/polymer in the DNA complex, there would be no need for this anionic layer. Accordingly, the teachings of these references cannot be logically combined to render Claim 1 and its dependent claims *prima facie* obvious.

In summary, applicants' claimed process distinguishes over the primary reference, Semple et al., in that Claim 1 recites that the cationic surface potential of the DNA complex is converted to a neutral or net anionic surface potential by reacting the cationic lipids or the cationic polymers <u>in the DNA complex</u> with a reagent selected from the group consisting of citraconic anhydride and N-hydroxysuccinimide acetate. In contrast, Semple et al. converts the cationic surface potential by changing the pH of the environment. The secondary references Trubetskoy et al. and Monahan et al. do not cure this deficiency because they do not disclose reacting a reagent with lipids/polymers <u>already complexed with DNA</u>. Moreover, there is no motivation to combine such references because one of skill in the art would have no desire to do so as each reference discloses its own unique approach to addressing the problem of a cationic surface potential other than the approach recited in Claim 1.

Given the above, Semple et al., Monahan et al., and Trubetskoy et al. do not render applicants' claims obvious and the Examiner's Section 103 rejection of Claims 1, 7, 11, 14, 15, 18 to 23, and 28 to 30 based thereon should be withdrawn.

Discussion of the Examiner's Indefiniteness Rejection

The Examiner rejected Claims 11, 14, 15, 21 to 23, 25 to 27, 30 and 32 under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner asserts that the phrase "said complex" in Claims 11, 14, 15, 21 to 23, 25 to 27, 30 and 32 is vague and indefinite since it is unclear which complex applicants are referring since claim 1 recites "a complex" twice. Applicants have amended claim 1 to recite the phrase "a complex" only once.

The Examiner also asserts that the phrase "wherein said complex further comprises a targeting ligand covalently attached to a cationic lipid or polymer" in Claims 11, 21, and 25 is vague and indefinite since it is unclear if the targeting ligand is covalently attached to an additional cationic lipid or polymer or to the cationic lipid or polymer in the complex of the colloid recited in Claim 1. Applicants have amended Claims 11, 21, and 25 to recite "said cationic lipid or cationic polyer" to make clear that the targeting ligand is covalently attached to the cationic lipid or polymer in the DNA complex recited in Claim 1.

Given the amendments to the claims as discussed above, the indefiniteness rejections should be withdrawn.

Conclusion

In view of the foregoing amendments and remarks, applicants assert that the claims are in condition for allowance, and request respectfully issuance of a Notice of Allowance. As already discussed with the Examiner by phone on April 10, 2007, the undersigned requests an in-person interview prior to the issuance of an action.

This Reply is being filed along with a Petition for a one-month extension of time along with the associated fee. If any other fees are required in order to continue the prosecution of this application, the Office is authorized to charge such fees to Deposit Account 19-5425.

Respectfully submitted,

/Marc S. Segal/

Marc S. Segal
Reg. No. 40,163
Synnestvedt & Lechner LLP
1101 Market Street, Suite 2600
Philadelphia, PA 19107
Telephone: (215) 923-4466
Facsimile: (215) 923-2189